EFFECTS OF PROLONGED CENTRIFUGATION ON GROWTH

AND ORGAN DEVELOPMENT OF RATS

By Jiro Oyama and William T. Platt

Environmental Biology Division
NASA, Ames Research Center
Moffett Field, California

Running Title: Centrifugation on Growth of Rats

Mailing Address for Proof: Jiro Oyama
Environmental Biology Division
NASA, Ames Research Center
Moffett Field, California

[redacted]

ABSTRACT

Mature and weanling Sprague-Dawley female rats were centrifuged at 2.5, 3.5, and 4.7 G for periods of time ranging up to 1 year. The growth rates and final body weights of weanling rats were significantly lower than those of noncentrifuged control rats. Both mature and weanling rats experienced initially a temporary loss in body weight due to inanition and reduced food consumption. Comparison of organ/body weight ratios of 4.5-month and 1-year centrifuged rats with corresponding control rats indicated that prolonged exposures caused only a few changes. Relative weights of the adrenals of 4.5-month centrifuged rats were reduced while the livers of 1-year centrifuged rats were increased. There was an apparent decrease in red blood cells and a significant decrease in hematocrit values of centrifuged rats. No histopathology was found in any of the centrifuged groups of rats which could be attributed to the exposure treatment. Results of this study show that rats are able to tolerate prolonged periods of simulated high gravity environments with little, if any, serious deleterious effects.

INDEX TERMS

Prolonged centrifugation   growth   organ development   pathology

hematology   food consumption

[redacted]

The phenomenon of the weightless state in space flight has raised an array of interesting questions, heretofore largely overlooked, on the fundamental role and significance of gravity, per se, on growth, development, and metabolism of terrestrial organisms. Experimental studies of gravity effects on mammals have been largely concerned with various physiologic responses and the determination of tolerance limits of animals acutely exposed to simulated changes in gravity by centrifugation. The effects of prolonged centrifugation on growth of rats was first reported by Matthews (3) in 1953. Subsequently, other workers reported on various long-term exposure studies on chickens (8,9), mice and hamsters (1,2,12), and rats (4,5,6,10). The present study deals with the effects of different G-loads upon growth and organ development of rats centrifuged for various periods of time.

METHODS

Animals.- Female Sprague-Dawley rats were used in all studies. Except as otherwise noted, all rats were fed Purina laboratory chow and water *ad libitum*. Noncentrifuged rats (controls) were handled and maintained under conditions as close as possible to the centrifuged rats.

Centrifuge.- A ten radial armed centrifuge 8.5 ft in radius was used. Cages (20 × 10 × 10 in.) large enough to hold 4 to 6 rats were suspended by means of a pivotal yoke assembly from each arm positioned at one of 3 fixed distances (4, 6, or 8 ft) from the center of rotation. The cage assembly had one degree of freedom, swinging outward upon centrifugation. Each cage assumed a final position such that the resultant of the centrifugal and gravitational forces was vertical to the cage floor. The centrifuge was run at a constant 40 rpm. Animals were exposed to a resultant force of either 2.5, 3.5, or 4.7 G. All cages were illuminated with individual fluorescent lights automated to provide on-off cycling at 6 A.M. and 6 P.M., respectively. The centrifuge was run continuously except for daily stoppages of approximately 20 minutes for servicing and weighing the rats. The centrifuge was normally run continuously over the weekends without stopping.

Organ Weights.- Rats were fasted for 18-20 hr prior to their removal from the centrifuge and exsanguinated by heart puncture under pentobarbital anesthesia (40 mg/kg, i.p.). Organs were carefully excised, trimmed, and weighed, and appropriate sections were fixed in cold 10% neutral formalin.[1]

RESULTS

Effect on Growth.- The differential effects on growth rates of weanling rats exposed to 2.5, 3.5, and 4.7 G for 130 days are shown in Fig. 1. There was a significant decrement (approximately 10%) in the body weights of rats following the first day of centrifugation. This loss in body weight was recovered between the 5th and 8th day of exposure. During this initial exposure period, the rats exhibited a post-rotary nystagmus along with a characteristic side-to-side rolling movement of the head whenever the centrifuge was stopped. Similar responses have been reported in chickens subjected to centrifugation (11). With continued exposure of rats to centrifugation, the nystagmus and head response disappeared and the rats appeared normal and completely unaffected whenever subjected to deceleration. During the

---

[1]Histopathologic examination of these tissues was performed by Dr. I. Ernest Gonzalez, Department of Pathology, University of California Medical Center, San Francisco, California.

period immediately following the first week of exposure, the centrifuged rats grew at a rate similar to but less than corresponding control rats. After 4 months of exposure, the mean body weights of the centrifuged rats were significantly less than those of control rats and inversely proportional to the G-load imposed.

In a second growth study of weanling rats, individual body weight measurements were recorded during a 40-day period immediately following the centrifugation-induced weight-loss period. Regression coefficients of growth for these rats are presented in Table 1. The regression coefficients for the 3.5 and 4.7 G exposed rats were significantly less for the control rats. There was, however, no significant difference in the growth rates between the two centrifuged groups.

The general pattern of response to centrifugation of mature rats was very similar to that of weanling rats except that mature rats required considerably more time to recover from the initial stress effects of centrifugation. Mature rats, for example, weighing approximately 230 g lost an average of 36 g when centrifuged at 4.7 G and this weight loss was not recovered until the 34th day of exposure. Weanling rats similarly treated recovered their weight loss in less than 8 days. This initial weight loss experienced by centrifuged rats can be attributed to inanition and reduced food consumption.

Figure 2 shows the pattern of response in body weight and food consumption of mature rats when subjected to 4.7 G over a 25-day period. It can be seen that the precipitous fall in body weight of rats exposed to centrifugation can be substantially reproduced in noncentrifuged pair-fed rats. If the centrifugation was continued over a sufficiently long period, the food consumption rate of exposed rats was restored and eventually exceeded that of control rats. In a representative experiment, for example, the food consumption rates of rats centrifuged for 3 months at 2.5, 3.5, and 4.7 G were 18.5, 16.4, and 17.4 g/rat/day, respectively; control rats averaged 15.3 g/rat/day. Corresponding mean body weights were 224, 218, and 198 g for the centrifuged rats and 261 g for the control rats. If the food consumption rate were based on body weight, the differences between the centrifuged and control rats would be even greater.

Effect on Organ Weights.- The organ/body weight ratios of rats exposed to 2.5, 3.5, and 4.7 G for 4.5 months along with corresponding control rats are presented in Table 2. All rats were approximately 4 weeks old at the start of the experiment and weighed 83 ±1.4 g. They were from the same group of animals employed in the growth study of Fig. 1. For each organ, an analysis of variance technique was used to test the hypothesis of homogeneous group means. Whenever the resultant F-value was significant ($P < 0.05$), intergroup comparisons were made utilizing Scheffe's method (7) with a confidence coefficient of 95%. This method, though somewhat conservative, overcomes the problem of unequal variances due to unequal sample size and also gives an exact confidence level for the experiment as a whole. The only statistically significant changes found were a decrease in body weight and adrenal/body weight ratio of the 3.5 and 4.7 G groups of rats. All other organ/body weight ratios were within the normal range of values for control rats. There was an indication that the relative weights of the pituitary of these rats were decreased and the liver increased although the changes were not considered statistically significant ($P < 0.10$). Similar data for rats exposed to 1 year of centrifugation are presented in Table 3. A summary of the statistical analysis is given in Table 4. The most consistent alteration found was an increase in liver weights in the centrifuged groups of rats. The differences were significant when compared to either the weight (Group A) or age (Group B) control rats. It should be noted that although the weight control rats were 3 months old and the age control rats more than a year older, no difference between these two control groups in liver/body weight ratios was found.

The increase in lung size of the 4.7 G centrifuged group (Group E) over the age control (Group B) was especially striking. Two of the centrifuged rats had very enlarged chests along with an abnormal curvature of the thoracic vertebrae. The smaller thymus of the centrifuged rats of groups (C), (D), and (E) and age control rats (Group B) compared to the younger weight control rats (Group A) was not unexpected since it is known that the thymus involutes in animals with age. A marked depletion in body fat, similar to that found by Smith and Kelly for chickens (8), was noted in all centrifuged rats whether exposed for 4.5 months or 1 year. For each exposure period, the depletion appeared to be proportional to the imposed G-load.

Hematology.- The hematologic response of rats centrifuged for 4.5 months and 1 year is shown in Table 5. There was an apparent decrease in the RBC and hematocrit values of 4.5-month centrifuged rats, but only the hematocrit values of the 2.5 and 4.7 G rats were statistically significant.

In the 1-year exposure study no direct comparison with control values could be made since hematologic studies were not run on 1-year age control rats. The values, however, for the 1-year centrifuged rats are quite similar to those of the 4.5-month exposed rats. WBC levels appeared to be decreased in the centrifuged rats of the 4.5-month study; however, the results were not statistically significant ($P < 0.10$). Rats centrifuged for 1 year at 3.5 G and then removed from the centrifuge for 30 days showed an increase in both RBC and hematocrit values compared to a comparable group of centrifuged rats on which hematology was done immediately after the year-long centrifugation.

Histopathology.- Detailed examination of tissues from rats subjected to centrifugation for periods of 4.5 months or 1 year revealed no consistent histopathologic change which could be attributed to the exposure. One of the control rats of the 1-year study, however, developed a spontaneous mammary tumor and was excluded from the experiment.

Results of histochemical studies of lipid distribution[2] in the various zones of the adrenal cortex were variable and showed no consistent pattern of alteration that could be attributed to the centrifugation treatment.

Lungs from both control and centrifuged rats showed in some cases diffuse interstitial pneumonitis and atelectasis. In both rats previously mentioned as having enlarged pulmonary capacities, histologic examination of the lungs revealed diffuse atelectasis and compensatory enphysema.

DISCUSSION

From this study it is clear that rats are able to tolerate prolonged exposures of relatively high G-loads with no serious pathologic alterations resulting. It should be pointed out that there were no deaths at any of the G-loads and exposure periods employed in this study. During the initial

[2]Neutral lipids were stained with oil red O while tissue cholesterol was determined by Schultz's method.

period of exposure to simulated high gravity conditions by centrifugation, rats are most adversely affected as indicated by their marked weight loss and reduction in food consumption. This initial stress response can be attributed not only to the imposed G-load but to the effects of angular acceleration. The influence of the latter factor is evident from the nystagmus response elicited in the rats during the first week of centrifugation. The relative contributions of the centrifugal force and of angular acceleration have not been assessed as yet; however, studies are in progress to resolve these components.

The general appearance and behavior of rats which have adapted to high-G environments were similar to noncentrifuged control rats; this is particularly true for rats exposed to 2.5 and 3.5 G. At 4.7 G, the tolerance limit is apparently reached and these rats were somewhat emaciated and seedy in appearance. From motion pictures taken of high-G adapted rats during centrifugation, it was evident that the rats could move about in a normal manner during centrifugation with no difficulty and were not adversely affected during either acceleration or deceleration.

It was previously reported (6) that when rats were centrifuged for 3 months and then removed from the centrifuge, their body weight increased rapidly from 6-11% during the first 24 hr off the centrifuge. This increase could be attributed to increased food and water consumption and concomitant rehydration. In contrast, rats in this study which were centrifuged for 1 year did not show any increase in body weight during the first 24 hr off the centrifuge. The 4 rats, centrifuged for 1 year at 3.5 G and then removed from the centrifuge for 30 days (Table 5), weighed an average of 282 g prior to removal, 279 g after the first 24 hr off the centrifuge, and 297 g after 30 days. The food consumption rate fell from 16.9 g/rat/day during centrifugation to 12.4 g/rat/day during the first 24 hr off the centrifuge. The food consumption rate after the first day was only slightly higher than the rate on the centrifuge but the water consumption rate, however, did increase from 31.6 ml/rat/day to 48.9 ml/rat/day. The reason for the difference in response upon removal from the centrifuge of the 3-month and 1-year exposed groups of rats is not clear. It is possible that in the 1-year exposure study, the almost daily stoppages of the centrifuge for servicing may have conditioned the rats so that they were unaffected when returned to normal gravity conditions.

The specific effects of intermittent stoppages of the centrifuge upon the growth patterns of exposed rats cannot be ascertained at present. The resolution of this problem will require the use of a fully automated centrifuge which can operate continuously without stoppage for animal servicing.

ACKNOWLEDGEMENT

The authors thank Miss Rosa Medina for her technical assistance and Mr. Eugene Averkin of the Computation and Analysis Branch for his assistance in the statistical analyses.

REFERENCES

1. Bird J. W. C., C. C. Wunder, N. Sandler, and C. H. Dodge. Analysis of muscular development of mice at high gravity. Am. J. Physiol. 204:523-526, 1963.

2. Briney, S. R., and C. C. Wunder. Comparative study of effects of gravity on the growth of hamsters and mice. Proc. Iowa Acad. Sci. 67:495-500, 1960.

3. Matthews, B. H. C. Adaptation to centrifugal acceleration. J. Physiol. 122:31, 1953.

4. Neville, E. D., and D. D. Feller. Lipid synthesis in livers of rats exposed to acceleration. Fed. Proc. 23:271, 1964.

5. Oyama, J., W. T. Platt, and E. Ogden. Exposure of rats to acute and chronic acceleration stress. Fed. Proc. 23:355, 1964.

6. Oyama, J., and W. T. Platt. Effects of deceleration on rats exposed to prolonged centrifugation. Nature 203:766-767, 1964.

7. Scheffe, H. The Analysis of Variance (3rd ed). New York: John Wiley and Sons, 1963, pp. 55-146.

8. Smith, A. H., and C. F. Kelly. Influence of chronic acceleration upon growth and body composition. Annals. N. Y. Acad. Sci. 110:410-424, [illegible].

9. Smith, A. H., C. M. Winget, and C. F. Kelly. Growth and survival of birds under chronic acceleration. Growth 23:97-108, 1959.

10. Steel, F. L. D. Early growth of rats in an increased gravitational field. Nature 193:583-584, 1962.

11. Winget, C. M., and A. H. Smith. Quantitative measurement of labyrinthine function in the fowl by nystagmography. J. Appl. Physiol. 17:712-718, 1962.

12. Wunder, C. C., S. R. Briney, M. Kral, and C. Skaugstad. Growth of mouse femurs during continual centrifugation. Nature 188:151-152, 1960.

TABLE 1.- Regression coefficients for growth of weanling rats subjected to centrifugation

Treatment	No. of rats	$\log_e A$	b ± standard deviation	P
Control	(48)	4.523	0.02381±0.00057	---
3.6 G	(24)	4.402	0.02131±0.00060	< 0.01
4.7 G	(24)	4.430	0.02074±0.00099	< 0.025

Exponential growth equation: $\log_e W = \log_e A + bX$

where W = body weight in grams

b = regression coefficient ± standard deviation in g/day/g body weight

X = days centrifuged

P value: Significant from control at stated probability.

TABLE 2.- Organ/body weight ratios of rats centrifuged for 4.5 months

GROUP	Age control	2.5 G	3.5 G	4.7 G
NO. OF RATS	5	4	4	4
AV. BODY WT, g	261±11	216±9	211±10*	205±14*
ORGAN (mg/100 g body wt)				
Liver	2,560±44	2,890±220	3,320±236	3,010±192
Kidneys, pr	695±18	685±27	720±27	732±28
Spleen	213±7	224±19	230±9	189±14
Thymus	108±13	111±21	103±15	56±16
Thyroid	206±10	166±20	134±23	163±38
Pituitary	5.87±0.26	4.98±0.15	4.24±0.48	4.83±0.62
Adrenals, pr	28.9±0.8	25.5±1.5	23.1±0.9*	21.6±1.2*
Ovaries, pr	49.5±3.4	48.0±5.0	47.7±9.4	51.0±3.2
Uterus	152±20	103±36	113±65	85.5±34
Lungs	419±13	428±23	446±16	483±32
Heart	299±3.6	336±19	356±40	371±25
Femur, left	376±11	409±21	383±19	393±85

Values are means ± standard error.

*Significant from control at a probability of 0.05 or less.

TABLE 3.- Organ/body weight ratios of rats centrifuged for 1 year

GROUP*	A. Control	B. Control	C. 3.5 G	D. 4.7 G	E. 4.7 G
NO. OF RATS	10	6	9	9	6
AV. BODY WT, g	250±6	323±13	272±8	231±8	262±13
ORGAN (mg/100 g body wt)					
Liver	2,770±90	2,640±50	3,390±90	3,280±110	3,240±240
Kidneys, pr	737±23(9)	702±22	741±29	759±27	800±29
Spleen	185±7	176±16	222±9	185±9	164±19
Thymus	101±9	34.2±4.9	34.6±4.4(8)	39.5±3.7	20.6±3.5
Thyroid	202±8	188±12	181±9	179±5	178±4
Pituitary	5.06±0.29	4.99±0.38	6.56±0.69	6.17±0.35	5.89±0.58
Adrenals, pr	24.2±1.0	22.4±2.2(5)	20.4±0.5	22.6±0.9	24.0±1.9
Ovaries, pr	434±29(8)	429±45	610±62(3)	510±26(4)	484±59
Uterus	175±13(8)	156±17	183±11(3)	194±16(4)	231±51
Lungs	498±29(4)	391±20	493±28(3)	479±20(3)	520±23(5)
Heart	351±15(6)	322±7	365±11(3)	336±12(4)	352±20
Femur	346±9	362±16	336±4(3)	378±14(4)	389±14

Values are means ± standard error for the number of rats in each group; when less, the number employed is given in parentheses.

*A. Weight controls, 3 months old; B. Age controls, 15.5 months old; C. Centrifuged for 348 days from weanling age (4 weeks old, av wt = 76.5 ± 1.3 g); D. Centrifuged for 348 days from weanling age (4 weeks old, av wt = 73.8 ± 1.1 g); E. Centrifuged for 89 days at 3.5 G followed by 295 days at 4.7 G. Rats were 3 months old (av wt = 237 ± 5 g) at start of centrifugation, 15.5 months at time of sacrifice.

TABLE 4.- Analysis of variance and group intercomparison of the means for selected organ/body weight ratios of rats centrifuged 1 year

Organ	F-ratio	Degrees of freedom	Pooled standard deviation	Group* comparisons, difference of means									
				B - A	B - C	B - D	B - E	A - C	A - D	A - E	C - D	A - C, D,E	B - C, D,E
Body wt	12.56†	(4,35)	25.77	73‡	51‡	92†	61†	-22	19	-12	41‡	-5.1	68†
Liver	7.536†	(4,35)	342.12	-134	-755†	-646‡	-602†	-621†	-512‡	-467	109	-533†	-667†
Lungs	5.215‡	(4,16)	51.29	-107†	-102	-88	-129†	5	19	-22	14	1.2	-106‡
Thymus	30.48‡	(4,34)	17.01	-67†	-0.4	-5.2	14	66†	61‡	80†	-4.8	69†	2.7

*Groups designated correspond to those listed in Table 3.

†Significant $P < 0.01$

‡Significant $P < 0.05$

TABLE 5.- Hematologic data on centrifuged rats

Exposure time	G	No. of rats	RBC, $10^6/mm^3$	Hematocrit, %	WBC, $10^3/mm^3$
4.5 months	2.5	4	7.81±0.16	42.4±0.8*	2.78±0.60
	3.5	4	7.46±0.14	42.6±0.6	4.61±1.26
	4.7	4	7.50±0.16	41.6±0.5*	3.46±0.68
	Control	5	8.18±0.26	45.1±0.7	5.90±0.49
1 year	3.5	9	8.23±0.29	41.8±0.9	4.38±0.53
	4.7	9	7.46±0.32	40.3±1.2	3.83±0.42
	3.5†	4	10.03±0.28	43.0±0.8	4.53±1.3‡

Values are means ± standard error.

*$P < 0.05$

†Centrifuged for 1 year and then removed from centrifuge for 30 days.

‡3 rats

FIGURE TITLES

Fig. 1.- Growth of female weanling rats under varying G-loads as a function of exposure time. Each point represents the mean body weight of 12 rats except for the noncentrifuged control group (1G) which is the mean of 36 rats.

Fig. 2.- Effect of centrifugation on body weight and food consumption of mature female rats. Each point represents the mean of 6 rats.

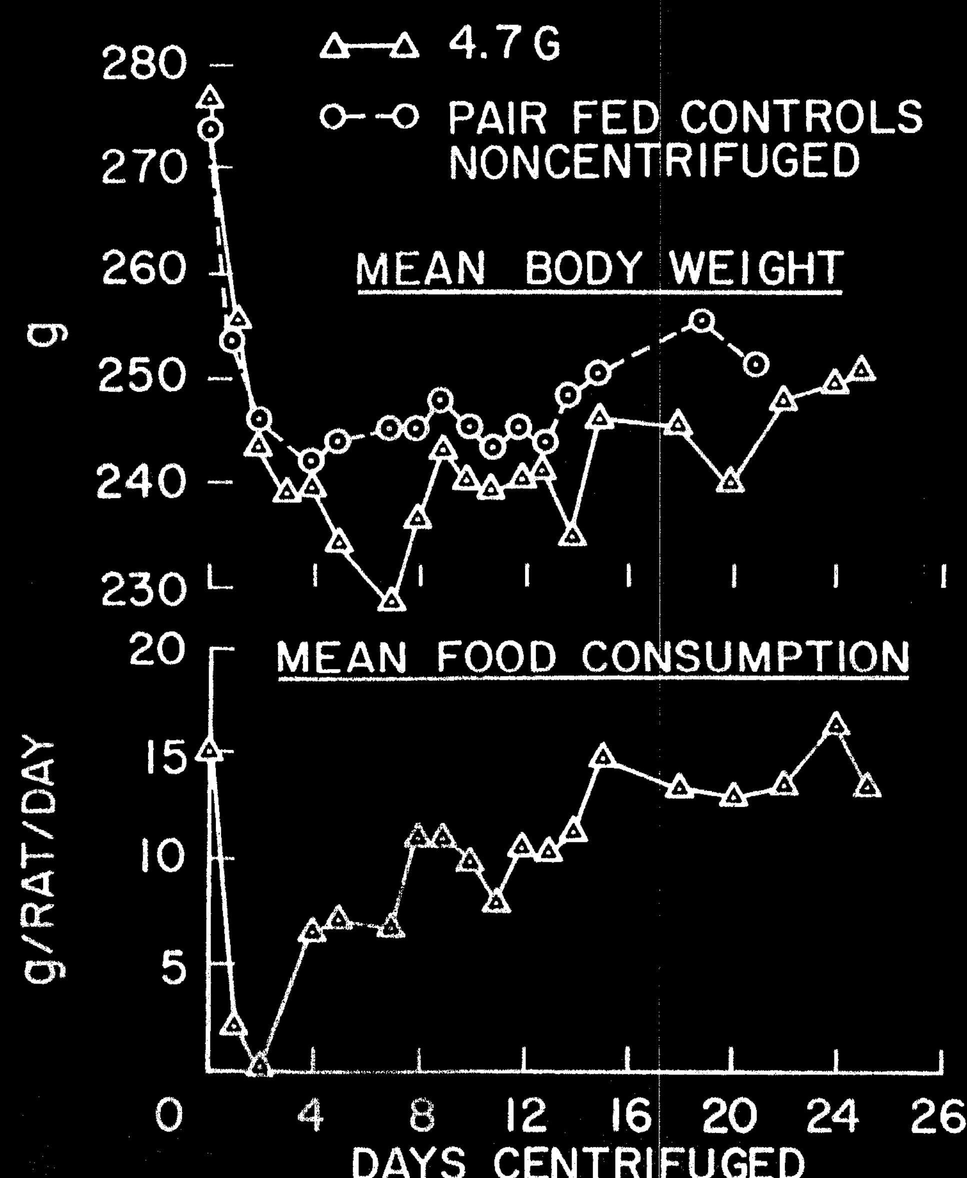

Fig. 2.- Effect of centrifugation on body weight and food consumption of mature female rats. Each point represents the mean of 6 rats.

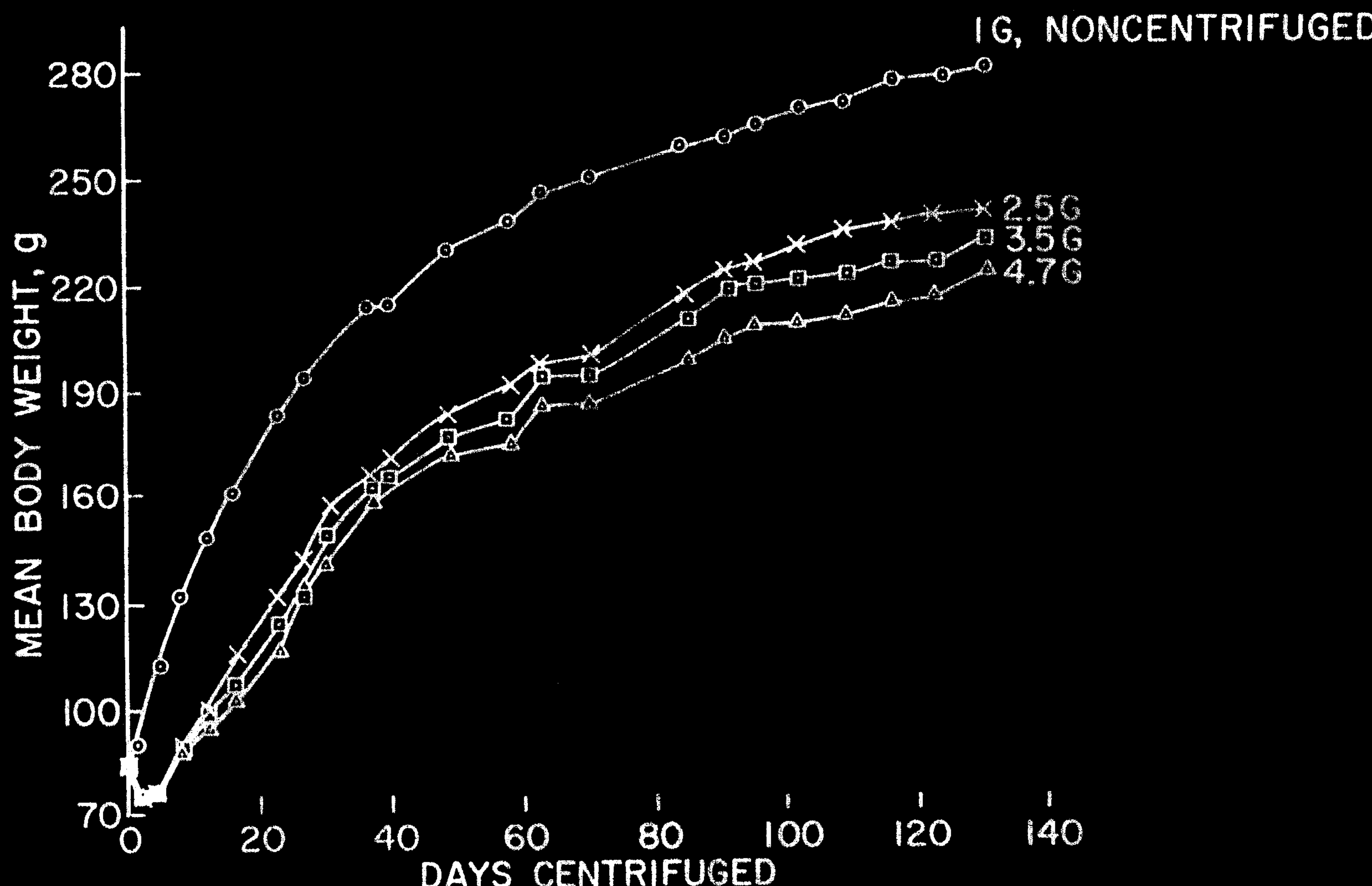

Fig. 1.- Growth of female weanling rats under varying G-loads as a function of exposure time. Each point represents the mean body weight of [illegible] rats except for the noncentrifuged control group (1G) which is the mean of [illegible] rats.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA